EXHIBIT F

Since rI1 does not contain an open reading frame, all *trans*-acting factors involved in the splicing reaction must be encoded in *C.reinhardtii*. So far, only a few mitochondrial splicing factors have been described for group II introns (reviewed in 52). In contrast, no chloroplast *trans*-acting factor able to bind group II introns is yet known. However, several mutants which affect chloroplast group II intron splicing have been reported; including two nuclear mutations in maize, which result in splicing deficiencies in chloroplasts (53). Although the plastome of *C.reinhardtii* does not contain continuous group II introns, there are two split introns (9). These introns are spliced *in trans* by forming the typical group II intron structure intermolecularly. At least 14 different nuclear products are required for *trans*-splicing of these introns (7,23,54,55). Some of these *trans*-splicing factors are probably also involved *in cis*-splicing of the heterologous intron rI1.

Intron RNA stability depends on the γ–γ′ interaction *in vivo*

Mutations of the γ–γ′ base pair, and to a lesser extent of the first intron nucleotide G1, result in an accumulation of very high levels of spliced intron RNA (when compared with the wild-type intron rI1). This accumulation of the wild-type intron in *C.reinhardtii* chloroplasts corresponds to the accumulation observed in *S.obliquus* mitochondria (56).

Since run-on transcription assays revealed that the rate of chimeric *tscA*-rI1 gene transcription remains unaltered in the mutants, accumulation of the excised intron must be due to increased intron stability. Similarly, in yeast mitochondria, several mutations of domains V and VI of aI5γ alter the accumulation of spliced intron (15,16). However, in contrast to our findings, the excised aI5γ intron barely accumulated. The mutants analyzed alter intron structure and splicing in a way that promotes the degradation of the excised intron RNA (44).

Intron mutations can also inhibit intron degradation, as shown for the first time with intron rI1. The altered sequence and structure of the intron RNA probably impedes either efficient recognition, or hydrolysis by ribonucleases. Alternatively, changes to the intron's structure could strengthen binding between the RNA and *trans*-acting proteins, providing the RNA with protection against RNases. This mechanism has previously been suggested for group I introns by Margossian and Butow (57). The nuclear-encoded SUV3 protein of yeast shows homology to helicases and is part of the mitochondrial exoribonuclease complexes 'mtEXO' (58,59). Mutation of the *suv3* gene leads to an increased accumulation of spliced group I introns in mitochondria, since SUV3 most probably releases intron-bound splicing proteins via its helicase function and thereby enables degradation of the naked RNA by the exoribonuclease complex (57,60). An efficient degradation of excised intron RNAs is important, since free introns can cause toxicity by interactions with other cellular RNAs or by exon reopening reactions (58,61).

The point mutations analyzed in this paper show dramatic effects not only on splicing efficiency, but also on exon ligation and intron stability. The conserved sequence of the γ–γ′ interaction, which in all group II introns has a purine at the γ site and a pyrimidine at the γ′ position, might be a compromise between these three functions. It has been conserved through evolution, since at least intron degradation depends not only on the γ–γ′ base pairing but also on the purine–pyrimidine distribution between these sites.

In most cases, all of the *cis*-acting elements mentioned above show similar effects on the relative splicing efficiency of rI1 both *in vitro* and *in vivo* as well as an intron aI5γ (15,34,35). However, further comparisons between autocatalytic splicing and *in vivo* processing of rI1 and other group II introns have revealed significant differences, mainly in phenotypes including post-splicing mechanisms. Since protein–RNA interactions determine splicing *in vivo*, intron-, species- and organelle-specific differences in binding between the intron RNA and *trans*-factors are likely. The co-evolution of introns and splicing factors has led to specific changes in intron sequences, structural elements and specific processing reactions. These changes have resulted in a complete loss of autocatalytic activity, as is the case for most group II introns (3,62). Still, a high similarity between group II introns is guaranteed, since the catalysis is driven by the RNA itself, and alteration of the intron RNA is therefore limited. This conservation enables a horizontal transfer of relatively ancient introns, which do not require specific splicing factors for processing. This is proven by the successful transfer of rI1 between mitochondria and chloroplasts of different green algae as well as into *Escherichia coli* (63).

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